

What is claimed:

1. A method of identifying binding sites on a macromolecule comprising:
 - (a) for at least one organic fragment (ORF), conducting, at separate values of parameter *B*, two or more simulated annealing of chemical potential calculations using the ORF as the inserted solvent; and
 - (b) comparing converged solutions from step (a) to identify first locations at which the relevant ORF is strongly bound, thereby identifying candidate sites for binding ligand molecules.
- 10 2. The method of claim 1, further comprising:
 - (c) identifying clusters of sites that strongly bind an ORF.
3. The method of claim 2, further comprising:
 - (d) conducting steps (a) and (b) for each of two or more ORFs and identifying clusters where two or more distinct ORFs bind.
- 15 4. The method of claim 3, wherein a cluster that binds three or more distinct ORFs is identified.
- 20 5. The method of claim 3, further comprising reducing the binding stringency in the vicinity of a cluster to further identify elements that would contribute to the binding of a bioactive agent.
- 25 6. The method of ^{claim 3}~~claim 1~~, further comprising:
 - (e) conducting, at separate values of a measure of chemical potential, two or more simulated annealing of chemical potential calculations using water as the inserted solvent;
 - (f) comparing converged solutions from step (c) to identify locations at which water is strongly bound, thereby identifying water locations which are not candidate sites for binding ligand molecules; and
 - (g) identifying first locations that are not water locations.

7. The method of claim 1, wherein the simulated annealing of chemical potential calculations comprise multiple steps of sampling, and wherein in a number of steps of the sampling the ORFs position is changed by a small amount and the resulting new position is accepted or rejected based on the change in energy as a result of the change attempted.

5

8. A method of identifying the chemical characteristics of compounds that bind a macromolecule comprising examining the functionalities and relative orientations of the ORFs found in a cluster pursuant to the binding site identifying method of claim 3.

10 9. A method of conducting combinatorial chemistry to identify compounds that interact with a macromolecule comprising:

(a) identifying classes of reactants that are modeled by the functionalities of the ORFs found in a cluster pursuant to the binding site identifying method of claim 3;

15 (b) designing a combinatorial synthetic protocol that calls for two or more synthetic procedures that react reagents of at least two of the classes identified in step (a); and

(c) conducting the combinatorial synthetic protocol to create candidate binding molecules.

20

10. A method of conducting a bioactive agent discovery process comprising:

(a) from a group of established combinatorial synthetic protocols or collections of chemical compounds or pools of chemical compounds, identifying those members of the group that provide a high density of compounds that meet for a macromolecule selection criteria identified from the binding site identifying method of claim 3; and

(b) conducting binding or functional assays to identify compounds obtained from the identified collections or protocols which bind or affect the function of the macromolecule.

25

30

ndb AJ